PATENT
Docket No. 377882001420

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Dated: 4/5/04 Signature: _____ (Grace Yu)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Gary VAN NEST et al.

Serial No.: 09/927,422

Filing Date: August 10, 2001

For: BIODEGRADABLE IMMUNOMODULATORY FORMULATIONS AND METHODS OF USE THEREOF

Examiner: N. M. Minnifield

Group Art Unit: 1645

**DECLARATION OF GARY VAN NEST, PH.D.**
**PURSUANT TO 37 C.F.R. § 1.132**

Commissioner for Patents
P.O. Box 1450, Alexandria
VA 22313-1450

Dear Sir:

I, Gary Van Nest, Ph.D., declare as follows:

1. I currently reside at 639 Skyline Drive, Martinez, California 94553.

2. I am an inventor named in the above-referenced patent application.

3. Described herein are results from experiments, performed by me or under my direction, which demonstrate the immunomodulatory activity of a phosphodiester polynucleotide as well as a phosphorothioate polynucleotide, when part of an immunomodulatory polynucleotide/microcarrier (IMP/MC) complex.

4. Polynucleotides were tested for immunomodulatory activity alone or as IMP/MC complexes in a human peripheral blood mononuclear cell (PBMC) assay. The human PBMC assay was performed as described in Example 4 of the specification and the IMP/MC complexes were prepared with cationic PLGA microspheres as described in Example 5 of the specification. Polynucleotides were made with phosphorothioate (P=S) or phosphodiester (P=O) linkages and tested as single agents or in complexes with PLGA microspheres. The polynucleotide sequences tested were: 5'-C, G-3' containing polynucleotide: TGACTGTGAACGTTCGAGATGA (SEQ ID NO:1); control (no CG) polynucleotide: TGACTGTGAACCTTAGAGATGA (SEQ ID NO:10); and control (no CG) polynucleotide: TGCTTGCAAGCTTGCAAGCA. The formulation of the polynucleotides tested were: SEQ ID NO:1 P=S, single-stranded; SEQ ID NO:1 P=O, single-stranded; SEQ ID NO:1 P=O, double-stranded; and controls SEQ ID NO:10 P=S, single-stranded or TGCTTGCAAGCTTGCAAGCA, P=S, single-stranded.

5. Immunomodulatory activity of the polynucleotides and complexes was determined by measuring IFN-γ and IFN-α concentration in the culture media after the cells were incubated with the test agents as described in the Examples of the specification.

6. Results from assays using PBMCs from a total of eight different donors are herein presented in Tables 1 and 2 of Exhibit A. Each table contains the mean result of four donors. As these results show, the phosphodiester polynucleotide in the MC complex was roughly comparable in activity or more active than the phosphorothioate polynucleotide in the MC complex. The IMP/MC complex made with the phosphodiester polynucleotide was active in inducing both IFN-α and IFN-γ despite the phosphodiester polynucleotide being inactive when used alone. For example, as shown in Table 2, the IMP/MC containing single-stranded SEQ ID NO:1 P=O stimulated 17,517 pg/ml IFN-α and 43 pg/ml IFN-γ even though alone single-stranded SEQ ID NO:1 P=O stimulated no IFN-α or IFN-γ. In the same experiment, the IMP/MC containing single-stranded SEQ ID NO:1 P=S stimulated 2159 pg/ml IFN-α and 537 pg/ml and the polynucleotide alone single-stranded SEQ ID NO:1 P=S stimulated 2069 pg/ml IFN-α and 147 pg/ml.

7. The experimental data presented herein demonstrate that polynucleotides containing either phosphorothioate or phosphodiester linkages in MC complexes are effective in stimulating IFN production from human PBMCs. In the context of such MC complexes, the phosphodiester polynucleotide is apparently protected from degradation so that successful

delivery to the target cell and subsequent stimulation occurs.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

March 30, 2004
Date

Gary Van Nest

EXHIBIT A

Table 1

| | IFN-α (pg/ml) | | IFN-γ (pg/ml) | |
|---|---|---|---|---|
| Stimulus | PN | IMP/MC | PN | IMP/MC |
| Cells alone | 89 | 102 | 2 | 2 |
| SEQ ID NO:1 P=S, single-stranded | 84 | 427 | 7 | 39 |
| SEQ ID NO:10 P=S, single-stranded (control) | 70 | 51 | 4 | 1 |
| SEQ ID NO:1 P=O, double-stranded | 66 | 2026 | 2 | 54 |
| SEQ ID NO:1 P=O, single-stranded | 51 | 965 | 2 | 29 |

| **Above results with background subtracted** | IFN-α (pg/ml) | | IFN-γ (pg/ml) | |
|---|---|---|---|---|
| Stimulus | PN | IMP/MC | PN | IMP/MC |
| Cells alone | 0 | 0 | 0 | 0 |
| SEQ ID NO:1 P=S, single-stranded | 0 | 325 | 5 | 37 |
| SEQ ID NO:10 P=S, single-stranded (control) | 0 | 0 | 3 | 0 |
| SEQ ID NO:1 P=O, double-stranded | 0 | 1924 | 0 | 52 |
| SEQ ID NO:1 P=O, single-stranded | 0 | 863 | 0 | 27 |

Table 2

| | IFN-α (pg/ml) | | IFN-γ (pg/ml) | |
|---|---|---|---|---|
| Stimulus | PN | IMP/MC | PN | IMP/MC |
| Cells alone | 26 | 452 | 29 | 9 |
| SEQ ID NO:1 P=S, single-stranded | 2095 | 2611 | 176 | 546 |
| TGCTTGCAAGCTTGCAAGCA, P=S, single-stranded (control) | 26 | 26 | 70 | 30 |
| SEQ ID NO:1 P=O, double-stranded | 26 | 16946 | 23 | 65 |
| SEQ ID NO:1 P=O, single-stranded | 26 | 17969 | 21 | 52 |

| **Above results with background subtracted** | IFN-α (pg/ml) | | IFN-γ (pg/ml) | |
|---|---|---|---|---|
| Stimulus | PN | IMP/MC | PN | IMP/MC |
| Cells alone | 0 | 0 | 0 | 0 |
| SEQ ID NO:1 P=S, single-stranded | 2069 | 2159 | 147 | 537 |
| TGCTTGCAAGCTTGCAAGCA, P=S, single-stranded (control) | 0 | 0 | 41 | 21 |
| SEQ ID NO:1 P=O, double-stranded | 0 | 16494 | 0 | 56 |
| SEQ ID NO:1 P=O, single-stranded | 0 | 17517 | 0 | 43 |

PN = polynucleotide alone
IMP/MC = polynucleotide - microsphere complex
P=S phosphorothioate linkage
P=O phosphodiester linkage